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Award Number: W81XWH-04-1-0624

TITLE: New Imaging Kit for Assessment of Estrogen Receptors with Single Photon Emission Computed Tomography

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REPORT DATE: March 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
March 2006	Final	15 Aug 04 – 14 Feb 06

New Imaging Kit for Assessment of Estrogen Receptors with Single Photon Emission Computed Tomography

5a. CONTRACT NUMBER

5b. GRANT NUMBERW81XWH
W81XWH-04-1-0624

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas
MD Anderson Cancer Center
Houston, Texas 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Purpose: To evaluate the feasibility of using 99mTc-glutamate peptide-estradiol (GAP-EDL) in imaging estrogen receptor positive (ER +) diseases. Methods: 3-Aminoethyl estradiol (EDL) was conjugated glutamate peptide (GAP) to yield GAP-EDL. Cellular uptake studies of 99mTc-GAP-EDL were conducted in ER (+) cell lines (MCF7, 13762 and T47D). To demonstrate whether GAP-EDL increases MAP kinase activation, Western blot analysis of GAP-EDL was performed in 13762 cells. Biodistribution was conducted in 13762 breast tumor-bearing rats at 0.5-4 hrs. Each rat was administered 99mTc-GAP-EDL (10 microCi/rat, 10 microgm/rat, iv). Two animal models (Rats and rabbits) were created to ascertain whether cellular or tumor uptake by 99mTc-GAP-EDL was via an ER-mediated process, In tumor model, breast tumor-bearing rats were pretreated with diethylstilbestrol (DES, n=3, 10 mg/kg, iv) 1 hr prior to receiving 99mTc-GAP-EDL (300 microCi/rat, iv). In endometriosis model, part of rabbit uterine tissue was dissected and grafted in the peritoneal wall. The rabbit was administered with 99mTc-GAP-EDL (1 mCi/rabbit, iv). Results: There was 10-40% decreased uptake in cells treated with DES or tamoxifen compared to untreated 99mTc-GAP-EDL. Western blot analysis showed an ERK1/2 phosphorylation process with GAP-EDL. Biodistribution studies showed that tumor uptake and tumor-to-muscle count density ratio in 99mTc-GAP-EDL groups were significantly higher than in 99mTc-GAP groups at 4 hrs. In 99mTc-GAP-EDL, ROI analysis of images showed that tumor-to muscle ratios were decreased in blocking groups. In endometriosis model, the grafted uterine tissue could be visualized by 99mTc-GAP-EDL. Conclusion: A new imaging kit for assessment of estrogen receptors with single photon emission computed tomography (SPECT) was developed. Cellular or tumor uptake of 99mTc-GAP-EDL was via an estrogen receptor-mediated process. 99mTc GAP-EDL is a useful ER (+) imaging agent.

15. SUBJECT TERMS
Estrogen,Receptor, SPECT, Tc-99m, Estradiol

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	36	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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PROGRESS REPORT

NEW IMAGING KIT FOR ASSESSMENT OF ESTROGEN RECEPTORS WITH SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

(BCRP BC03298)

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ABSTRACT

Purpose: To evaluate the feasibility of using ^{99m}Tc-glutamate peptide-estradiol (GAP-EDL) in imaging estrogen receptor positive (ER +) diseases. **Methods:** 3-Aminoethyl estradiol (EDL) was conjugated glutamate peptide (GAP) to yield GAP-EDL. Cellular uptake studies of ^{99m}Tc-GAP-EDL were conducted in ER (+) cell lines (MCF7, 13762 and T47D). To demonstrate whether GAP-EDL increases MAP kinase activation, Western blot analysis of GAP-EDL was performed in 13762 cells. Biodistribution was conducted in 13762 breast tumor-bearing rats at 0.5-4 hrs. Each rat was administered ^{99m}Tc-GAP-EDL (10 microCi/rat, 10 microgm/rat, iv). Two animal models (Rats and rabbits) were created to ascertain whether cellular or tumor uptake by ^{99m}Tc-GAP-EDL was via an ER-mediated process, In tumor model, breast tumor-bearing rats were pretreated with diethylstilbestrol (DES, n=3, 10 mg/kg, iv) 1 hr prior to receiving ^{99m}Tc-GAP-EDL (300 microCi/rat, iv). In endometriosis model, part of rabbit uterine tissue was dissected and grafted in the peritoneal wall. The rabbit was administered with ^{99m}Tc-GAP-EDL (1 mCi/rabbit, iv). **Results:** There was 10-40% decreased uptake in cells treated with DES or tamoxifen compared to untreated ^{99m}Tc-GAP-EDL. Western blot analysis showed an ERK1/2 phosphorylation process with GAP-EDL. Biodistribution studies showed that tumor uptake and tumor-to-muscle count density ratio in ^{99m}Tc-GAP-EDL groups were significantly higher than in ^{99m}Tc-GAP groups at 4 hrs. In ^{99m}Tc-GAP-EDL, ROI analysis of images showed that tumor-to muscle ratios were decreased in blocking groups. In endometriosis model, the grafted uterine tissue could be visualized by ^{99m}Tc-GAP-EDL. **Conclusion:** A new imaging kit for assessment of estrogen receptors with single photon emission computed tomography (SPECT) was developed. Cellular or tumor uptake of ^{99m}Tc-GAP-EDL was via an estrogen receptor-mediated process. ^{99m}Tc GAP-EDL is a useful ER (+) imaging agent.

INTRODUCTION

The estrogen receptor (ER) is one of the most important factors to predict the prognosis or response to therapy in breast cancer. Estrogen receptor- positive (ER +) tumors have a more favorable prognosis than estrogen receptor-negative (ER -) tumors. Additionally, ER status determined the likely hood of response to hormonal therapy [1-3]. Until now, the presence of ERs was measured in vitro in a sample obtained at biopsy or resection of the tumor. In clinical practice, these assays are imperfect tools for guiding therapy; only 55%-60% of patients with ER (+) tumors and 8-10% of patients with ER (-) tumors respond to hormonal manipulation. In addition, tissue specimen biopsy is an invasive process and can determine only local neoplasm status. Owing to greater tumor specificity, radioscintigraphy is expected to be highly detectable examination for ER status. Such an imaging modality may improve the specificity and monitor the responsiveness of tumors to therapy for individual patients. Thus, we explored a novel method to develop a simple and efficient chelating chemistry. The excitatory amino acid glutamate (Glu) exerts its action via a variety of glutamate receptors (GluRs). It is known that poly-glutamate peptide (GAP, MW 1,000) stimulates bone resorption in vitro and specific to GluRs [4,5]. Because GAP is a targeted carrier, it would be suitable to conjugate estradiol (EDL) to GAP and GAP-EDL may bind to cytosolic ERs. With acid residue from GAP, GAP could chelate radiometallic isotopes for imaging and radiotherapeutic applications. This study is aimed to develop ^{99m}Tc-GAP-EDL to imaging estrogen receptor positive (ER +) diseases (breast cancer, endometriosis).

BODY

TASK 1. Radiosynthesis of an Analogue of Estradiol and in Vitro Pharmacological Evaluation- animal studies.

Chemistry

The structures of EDL and GAP-EDL were confirmed by proton-NMR spectrum (Fig. 2-3). There was 15% (weight by weight) EDL conjugated to GAP as determined by UV spectroscopy. Radiochemical purity of ^{99m}Tc-GAP-EDL was assessed by Radio-TLC scanner (Bioscan, Washington, DC) using 1M ammonium acetate: methanol (4:1) as an eluant. ^{99m}Tc-GAP-EDL showed 97% pure (Fig. 4).

In Vitro Cellular Uptake Studies

There was a marked increase in the uptake of ^{99m}Tc GAP-EDL as a function of ER compared with the uptake of ^{99m}Tc-GAP (Fig. 5-7). There was 10-40% decreased uptake in MCF-7 and T47D cells treated with diethylstilbestrol when compared to ^{99m}Tc-GAP-EDL (Fig. 5). There was 10% decreased uptake of ^{99m}Tc-GAP-EDL in MCF-7 cells treated with tamoxifen (Fig. 6). The findings indicated that cellular uptake of ^{99m}Tc-GAP-EDL was via an ER-mediated process.

Western blot analysis

Western blot analysis showed that estradiol (0.2nM) and GAP-EDL (1 nM) induced phosphorylation of ERK1/2 whereas tamoxifen (1 and 100nM) blocked phosphorylation of ERK1/2 (Fig. 8).

TASK 2. Determination the Dose and Time Effect of ^{99m}Tc-Estradiol

Tissue Distribution Studies

In vivo biodistribution studies showed that count density ratios for tumor-to-muscle was increased as a function of time in ^{99m}Tc-GAP-EDL groups. At 4 hours, tumor uptake, tumor-to-muscle and tumor-to-blood count ratios were significantly higher in ^{99m}Tc-GAP-EDL groups than in ^{99m}Tc-GAP groups (0.519±0.036 vs. 0.323±0.024, $p<0.05$, 7.923 ±0.560 vs. 6.504±1.670, $p<0.05$, and 0.719±0.202 vs. 0.549±0.015, $p<0.05$) (Tables 1 and 2). Uterus uptake, uterus-to-muscle and uterus-to-blood count ratios were also significantly higher in ^{99m}Tc-GAP-EDL groups than in ^{99m}Tc-GAP groups (0.504±0.020 vs. 0.188±0.038, $p<0.05$ 0.518±0.025 vs. 0.321±0.042, $p<0.05$ and 7.923±0.560 vs. 3.522±0.802, $p<0.05$).

TASK 3. ER (+) Disease Response to Therapy

Gamma Scintigraphy Imaging Studies in Tumor-Bearing Rats

In planar images of breast tumor-bearing rats, ROI analysis of images at 0.5-4 hrs showed that tumor-to muscle ratios were 1.67-2.95 and 1.26-1.75 for ^{99m}Tc-GAP-EDL and ^{99m}Tc-DTPA, respectively (Fig. 9). In blocking studies, tumor-to muscle ratios were 1.98-2.39 and 1.21-1.63 for ^{99m}Tc-GAP-EDL and blocked groups, respectively. There was a marked decrease in rats pretreated with diethylstilbestrol (Fig. 10).

Gamma Scintigraphy Imaging Studies in Rabbits with Endometriosis

Four endometriosis masses were implanted 8 weeks in advance on anterior abdominal wall, parallel to linea alba. Two grafts were macroscopically visible at 8 weeks. One implant was small and one showed as a visible cyst of ~1.5 cm^3. The cyst-like implant

correlated with increased radiotracer uptake. Increased activity inferior to the left kidney appeared when we used compression technique to empty the bladder which retrospectively established the presence of adhesion of uterus and ureter tissue (Fig. 11). Necropsy was performed 2.5 HR after injection time. Planar scintigraphy imaging of uterus, ovary and implants revealed increased uptake of ^{99m}Tc-GAP-EDL in comparison with surrounding abdominal wall tissue (Fig. 12).

KEY RESEARCH ACCOMPLISHMENTS

- **Chemistry**
    - EDL and GAP-EDL were synthesized and confirmed by proton-NMR spectrum (Fig. 2-3).
    - ^{99m}Tc-GAP-EDL was synthesized with acceptable purity (Fig. 4).
- **In Vitro Cellular Uptake Studies**
    - ^{99m}Tc GAP-EDL accumulated in ER (+) cells while ^{99m}Tc-GAP did not (Fig. 5-7).
    - Blocking of ^{99m}Tc GAP-EDL uptake was observed in MCF-7 and T47D cells treated with diethylstilbestrol (Fig. 5).
    - Blocking of ^{99m}Tc GAP-EDL uptake was observed in MCF-7 cells treated with tamoxifen (Fig. 6).
    - The findings indicated that cellular uptake of ^{99m}Tc-GAP-EDL was via an ER-mediated process.

- **Western blot analysis**
    - Estradiol and GAP-EDL (induced phosphorylation of ERK1/2 whereas tamoxifen blocked phosphorylation of ERK1/2 (Fig. 8).
- **Tissue Distribution Studies**
    - Count density ratios for tumor-to-muscle increased as a function of time in ^{99m}Tc-GAP-EDL groups.
    - At 4 hours, tumor uptake, tumor-to-muscle and tumor-to-blood count ratios were significantly higher in ^{99m}Tc-GAP-EDL groups than in ^{99m}Tc-GAP groups (Tables 1 and 2).
    - Uterus uptake, uterus-to-muscle and uterus-to-blood count ratios were also significantly higher in ^{99m}Tc-GAP-EDL groups than in ^{99m}Tc-GAP groups.
- **Gamma Scintigraphy Imaging Studies in Tumor-Bearing Rats**
    - ^{99m}Tc-GAP-EDL showed a significantly higher tumor-to-muscle ratio than ^{99m}Tc-DTPA (Fig. 9).
    - In blocking studies, tumor-to muscle ratios were decreased for ^{99m}Tc-GAP-EDL.
    - There was a marked decrease in ^{99m}Tc-GAP-EDL uptake in rats pretreated with diethylstilbestrol (Fig. 10).
- **Gamma Scintigraphy Imaging Studies in Rabbits with Endometriosis**
    - A cyst-like implant correlated with increased radiotracer uptake. (Fig. 11).
    - Imaging of uterus, ovary and implants revealed increased uptake of ^{99m}Tc-GAP-EDL in comparison with surrounding abdominal wall tissue (Fig. 12).

REPORTABLE OUTCOMES

- Presented at 91st Scientific Assembly and Annual Meeting of the Radiological Society of North America, Chicago, IL. Nov. 27- Dec. 2. 2005, by: Kim EE, Azhdarinia A, Inoue T, Oh C-S, Yang DJ. PET/SPECT targeted imaging of estrogen receptors with ^{99m}Tc- and ^{68}Ga-labeled estradiol. Radiology, 2005 (LPR12-09)
- Submitted to the European Journal of Nuclear Medicine and Molecular Imaging, Nobukazu Takahashi, David J. Yang, Saady Kohanim, Chang-Sok Oh, Dong-Fang Yu, Ali Azhdarinia, Xiaochun Zhang, Joe Y Chang, E. Edmund Kim. Targeted Functional Imaging of Estrogen Receptors with ^{99m}Tc-GAP-EDL.

CONCLUSIONS

In order to prolong DTPA-drug conjugates targeting potential, we used glutamate peptide (GAP) as a chelator for ^{99m}Tc. GAP was selected because it binds to glutamate or folate receptors [4,5]. Here we used glutamate peptides (GAP, MW. 1500-3000) with 10-20 acid moieties and found they are suitable for imaging. Similar to DTPA or EDTA, three acid moieties are reserved for ^{99m}Tc-chelation. The conjugation reaction between GAP and targeting agent could be conducted in aqueous (wet) or organic solvent (dry) conditions. Upon completion of conjugation reaction, the remaining acid moiety can easily be labeled with ^{99m}Tc.

We used three cell lines for in vitro studies. Two of which were human cell lines (MCF7 and T47D) and showed a there was 10-40% decreased uptake in MCF-7 and T47D cells

treated with diethylstilbestrol when compared to control. MCF-7 and T47D are the high ER (+) breast cancer cell lines. There was 10% decreased uptake of ^{99m}Tc-GAP-EDL in cells treated with tamoxifen in MCF-7 cells. Tamoxifen interferes with the activity of estrogen. The ability of spatial resolution of gamma camera imaging system was not enough to evaluate the small size tumor in nude mice. Thus, we used a rat tumor cell line (13762) for in vitro and in vivo studies. This cell line was derived from DMBA-induced mammary adenocarcinoma cells and considered as an ER (+) cell line [6]. In vitro cell culture studies showed that there was a marked increase in uptake of ^{99m}Tc GAP-EDL compared to ^{99m}Tc GAP.

In biodistribution and imaging studies with rats bearing 13762 breast cancer cells, tumor-to-muscle, uterus-to-muscle and uterus-to-blood count density ratios in ^{99m}Tc-GAP-EDL groups were significantly higher than in ^{99m}Tc-GAP groups at 4 hrs post-administration. ROI analysis of images showed that tumor-to muscle ratios were higher with ^{99m}Tc-GAP-EDL than with ^{99m}Tc-DTPA. In blocking studies, tumor-to muscle ratios were higher with ^{99m}Tc-GAP-EDL than with blocked groups.

To demonstrate ^{99m}Tc GAP-EDL binds to ERs and has could be used as a functional ER imaging agent, we have created an endometriosis using rabbit as a model. Endometriosis is associated with ER overexpression in uterine tissue. In our rabbit model, part of the uterine tissue was grafted to the peritoneal wall. Planar imaging studies showed that these grafts could be visualized by ^{99m}Tc GAP-EDL. Pathological examination supports the imaging findings. The in vitro and in vivo findings appear to support our hypothesis that ^{99m}Tc GAP-EDL binds to ERs and is a functional ER imaging agent.

ER modulators such as tamoxifen are important tools in researching the mechanisms of action of estrogen as well as in clinical practice [7]. Several recent reports have demonstrated that estrogen rapidly activate MAP kinases in a number of model systems [8-12]. Estradiol increases MAP kinase (MAPK) activation as indicated by ERK1 and ERK2 phosphorylation in MCF-7 cells, which in turn activates the nuclear factor kappa B (NFκB) signaling pathways as indicated by an increase in the p50 subunit of NFκB in nuclear extracts [8]. Our Western blot analysis showed that estradiol and GAP-EDL induced phosphorylation of ERK1/2 via MAPK in 13762 breast cancer cells. GAP-EDL may also be involved in MAPK pathway and subsequently involved in cell proliferation.

In summary, a new imaging kit for assessment of estrogen receptors with SPECT was developed. Our findings suggest that tumor uptake of ^{99m}Tc-GAP-EDL is via an estrogen receptor-mediated process. GAP-EDL increases MAPK activation as indicated by ERK1/2 phosphorylation. The dose and time Effect of ^{99m} Tc-GAP-EDL were determined. ^{99m}Tc-GAP-EDL has potential to improve diagnosis and prognosis, planning, and monitoring of ER positive diseases.

So What

A new imaging kit for the assessment of ER (+) disease has been developed. From biodistribution and imaging findings, the data indicate that this kit is useful for non-invasive detection of ER status. In addition, the kit provides a cost-effective approach for targeted imaging. Taken together, the data warrant further exploration of ^{99m}Tc-GAP-EDL in the clinical setting.

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APPENDIX

RSNA Abstract

Objective: The absence or presence of functional estrogen receptors (ER-α and ER-β) is an important predictor of breast cancer prognosis and plays an important role in the determination of proper treatment. The study is aimed to develop ^{99m}Tc- and ^{68}Ga-estradiol to diagnose and monitor ER (+) breast cancer.

Methods: 3- and 17-Aminoethyl estradiol (EDL) was synthesized by reacting estrone and bromoacetonitrile or sodium cyanide, followed by reduction with lithium aluminum hydride. 3- and 17-Aminoethyl estradiol was then conjugated glutamate peptide (GAP, MW. 1,500-3,000). ^{99m}Tc-pertechnetate was added to GAP-3-EDL or GAP-17-EDL and tin chloride (II). GAP-3-EDL and GAP-17-EDL were also labeled with $^{68}GaCl_3$. Cellular uptake was conducted in low and high ER (+) breast cancer cell lines (Low: 13762NF, High: MCF7 and T47D) incubated with labeled GAP-EDL (6μg/well, 1 μCi/well). In biodistribution and imaging studies, each animal was injected intravenously with ^{99m}Tc- and ^{68}Ga-GAP-EDL (10 μCi/rat, 10 μg/rat for biodistribution and 300 μCi/rat for imaging) and the data were collected at 0.5-4 hrs. To ascertain whether the tumor uptake by with ^{99m}Tc-GAP-EDL was related to estrogen receptors, rats was pretreated with diethylstilbestrol (n=3, 10 mg/kg, iv) 1 hr prior to receiving labeled GAP-EDL (300 μCi/rat, iv) and imaged at 0.5-4.0 hrs.

Results: There was 30% estradiol conjugated to GAP as determined by UV spectroscopy. The yield of ^{99m}Tc- and ^{68}Ga-GAP-EDL was 97% pure. No marked difference between position 3 and 17 GAP-EDL in cellular uptake (ave. 1-4%, 0.5-4hr incubation). There was 10-40% decreased uptake of ^{99m}Tc- and ^{68}Ga-GAP-3-EDL in cells treated with estrone. Radiolabeled GAP-3-EDL conjugates could be blocked with estrone or diethylstilbestrol. Biodistribution studies showed that tumor-to-tissue and uterine-to-tissue count density ratios in ^{99m}Tc-and ^{68}Ga-GAP-3-EDL groups were significantly higher than in GAP groups. In blocking studies, tumor-to muscle ratios were 1.98-2.39 and 1.21-1.63 for ^{99m}Tc-GAP-EDL and blocked groups, respectively. The findings suggest that tumor uptake of radiolabeled GAP-EDL is via an estrogen receptor-mediated process.

Conclusions: ^{99m}Tc- and ^{68}Ga-labeled estradiol may be useful in imaging functional ER (+) tumors and monitoring the responsiveness of tumors to chemotherapy.

ARRS Abstract

Kim EE, Yang DJ, Oh C, Azhdarinia **A.**. Differentiation of Tumor from Inflammation Using 99mTc- And 68Ga-EC Guanine. (accepted) ARRS 2006

Objective. DNA markers are useful to assess cell proliferation. The purpose of this study was to synthesize ^{99m}Tc- and ^{68}Ga-ethylenedicysteine-guanine (EC-Guan) for evaluation of cell proliferation by PET and SPECT.

Methods. Tumor cells were incubated with ^{99m}Tc- and ^{68}Ga-EC-Guan for confluence and cell cycle analysis. Prostate tumor cells that were overexpressing the HSV thymidine kinase gene, or various tumor cells were incubated with ^{99m}Tc- and ^{68}Ga-EC-Guan at 0.5-2 hrs. Thymidine incorporation assays were performed in lung cancer cells incubated with EC-Guan at 0.1-1 mg/well. Tissue distribution, autoradiography and planar scintigraphy of ^{99m}Tc- and ^{68}Ga-EC-Guan were determined in inflammation (by turpentine) and tumor-bearing rodents at 0.5-4 hrs.

Results. Cell culture assays indicated EC-Guan was incorporated in DNA S-phase, and there was no significant uptake difference between HSVTK overexpressed and normal groups. Biodistribution and scintigraphic imaging studies of ^{99m}Tc- and ^{68}Ga-EC-Guan showed increased tumor-to-tissue count density ratios as a function of time. There was much greater uptakes of labeled ED-Guan in tumor than inflammation. **Conclusion.** Our results indicate that ^{99m}Tc- and ^{68}Ga -EC-Guan are specific cell cycle-targeted agents which may be useful to assess tumor proliferation.

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Personnel Received Pay

Ali Azhdarinia

SUPPORTING DATA

Figure Legends

Fig 1. Synthetic Scheme of GAP-EDL

Fig 2. Proton NMR of EDL

Fig 3. Proton NMR of GAP-EDL

Fig 4. Radio-thin layer chromatographic analysis of ^{99m}Tc-GAP-EDL. Radiochemical purity of ^{99m}Tc-GAP-EDL was 97% using 1M ammonium acetate: methanol (4:1) as an eluant.

Fig 5. Cellular uptake of ^{99m}Tc -GAP-EDL in Human Breast Cancer Cells. There was 10-40 % significantly ($p<0.01$) decreased uptake of ^{99m}Tc-GAP-EDL when treated with diethylstilbestrol in MCF-7 and T47D cells.

Fig 6. Cellular uptake of ^{99m}Tc-GAP and ^{99m}Tc-GAP-EDL in MCF-7 Cells. Treatment tumor cells with tamoxifen showed significantly ($p<0.05$) 10% decreased uptake of ^{99m}Tc-GAP-EDL.

Fig 7. Cellular uptake of ^{99m}Tc –GAP and ^{99m}Tc -GAP-EDL in 13762 Cells. Both tracer uptake was gradually increased during 4 hours, however, the magnitude of ^{99m}Tc -GAP-EDL was significantly ($p<0.01$) higher than ^{99m}Tc-GAP at 2-4 hrs.

Fig 8. A representative Western blot is shown of phosph-ERK 1 and 2 in 13762 cell line after 3 min incubation with estradiol and GAP-EDL. Estradiol (0.2nM) and GAP-EDL (1 nM) induced phosphorylation of ERK1/2 whereas tamoxifen (1 and 100nM) blocked phosphorylation of ERK1/2.

Fig 9. Planar images of breast tumor-bearing rats after administration of ^{99m}Tc -GAP-EDL (left rat) and ^{99m}Tc -DTPA (right rat). A selected image is shown at 60 min post-injection. ^{99m}Tc -GAP-EDL showed high uptake, whereas ^{99m}Tc –DTPA had poor uptake in the tumor (arrows) ROI analysis showed tumor-to muscle ratios were 1.67-2.95 and 1.26-1.75 for ^{99m}Tc-GAP-EDL and ^{99m}Tc-DTPA, respectively

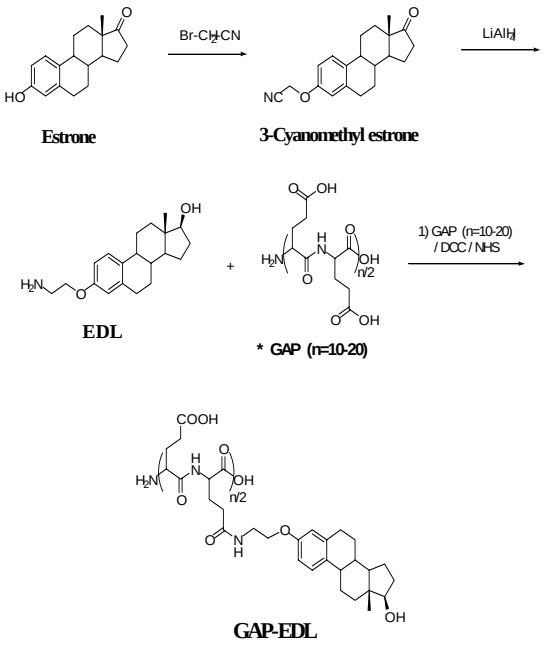
Fig 10. Planar scintigraphy images of a breast tumor-bearing rat pretreated with DES (10mg, iv, left) followed by ^{99m}Tc -GAP-EDL (0.3 mCi, iv). The image in panels a, b and c were as 15min, 60min and 60min post-administration. The rat pretreated with DES showed decreased uptake of ^{99m}Tc -GAP-EDL in comparison with the untreated rat. In blocking studies, ROI analysis showed that tumor-to muscle ratios were 1.98-2.39 and 1.21-1.63 for ^{99m}Tc-GAP-EDL and blocked groups. Arrows show tumors.

Fig 11. X-ray imaging (a) and planar scintigraphy of ^{99m}Tc -GAP-EDL in 30min and 120min post-administration of ^{99m}Tc-GAP-EDL (b and c) in an endometriosis

rabbit model. Arrows indicate the implanted sites of uterus tissues in panel-a. Two grafts were macroscopically visible at 8 weeks. As arrows indicated in panels b and c, the cystic implant was correlated with increased radiotracer uptake.

Fig 12. Necropsy was performed 2.5 HR after injection time. Photo of necropsy (a), graft implant with H and E stain (b), a photo of remaining uterus and an ovary and a graft implant (c) and planar image of the tissue containing uterus and an ovary and a graft implant (d) after necropsy. Implanted grafts revealed endometriosis by microscopic examination. Planar scintigraphy imaging of uterus, ovary and grafts reveals increased uptake of ^{99m}Tc -GAP-EDL.

Figures



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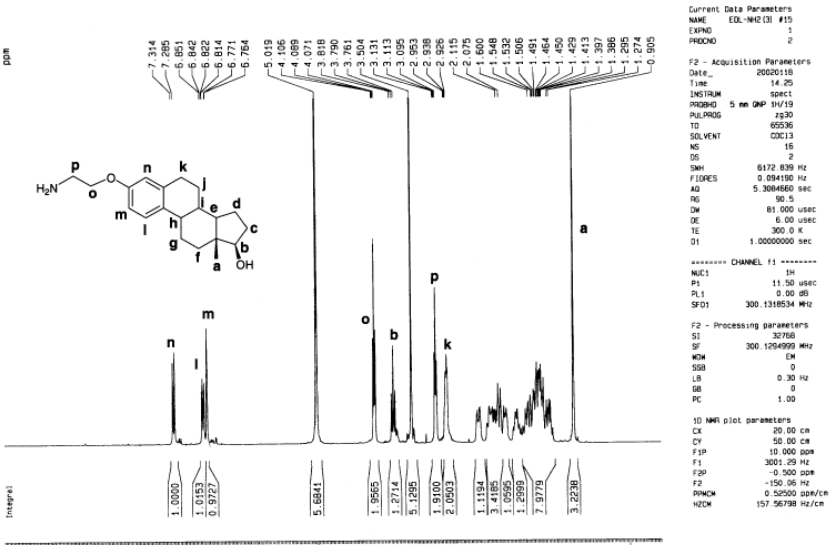
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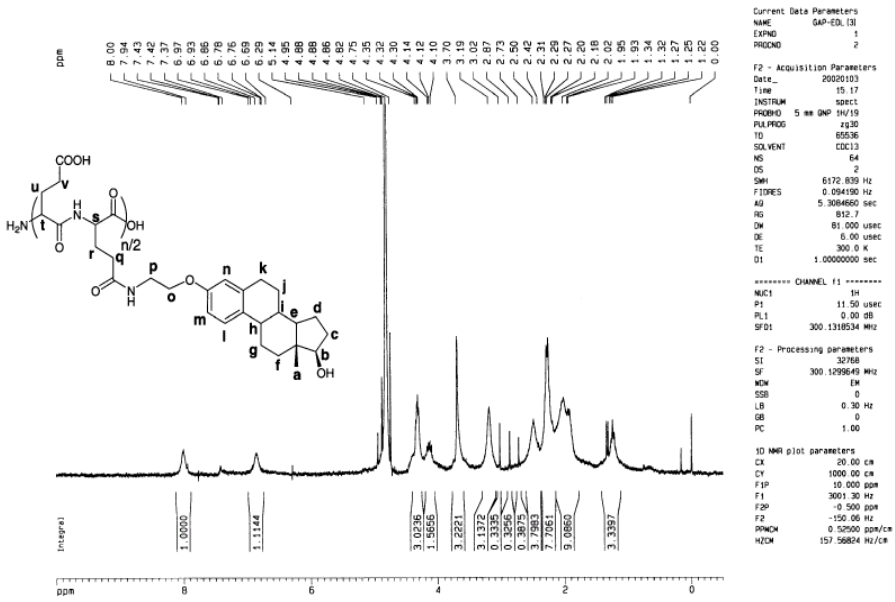
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n/2
OH
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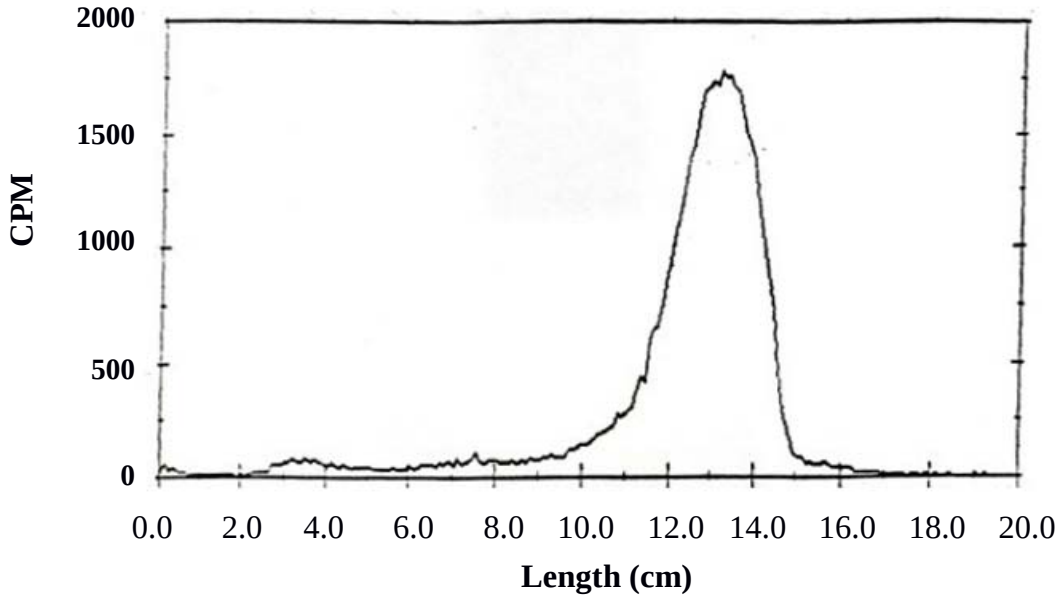
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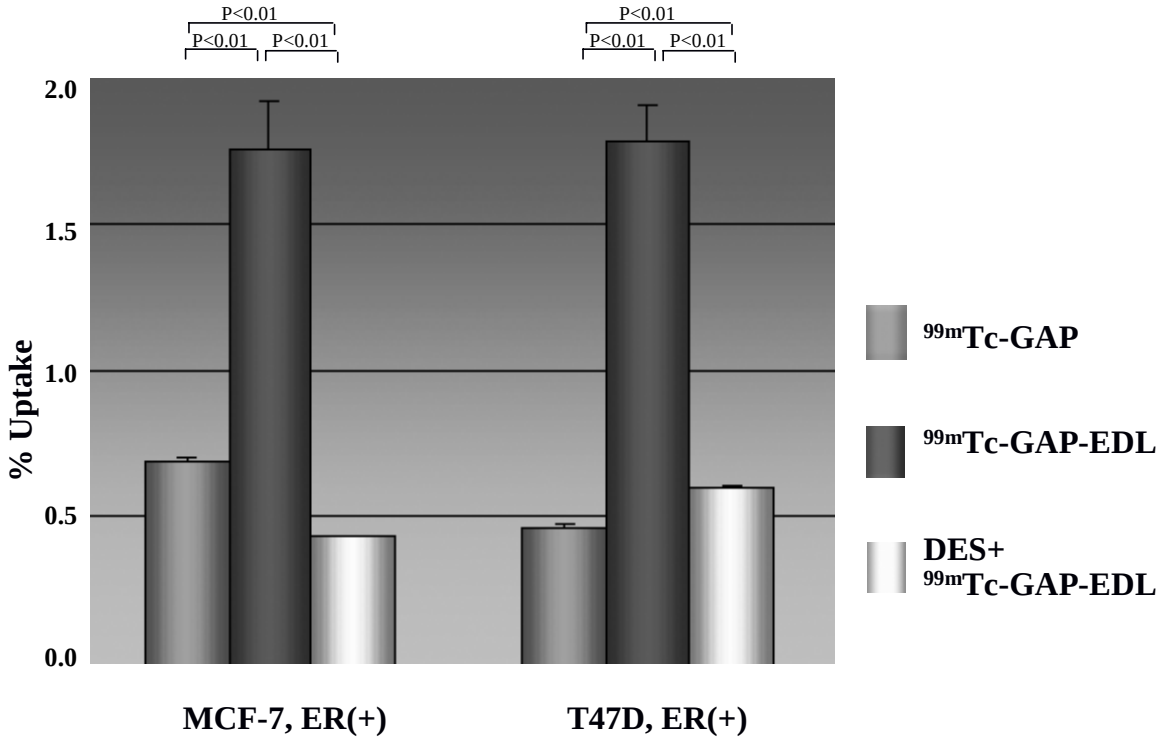
Length (cm)

Fig. 4.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% Uptake
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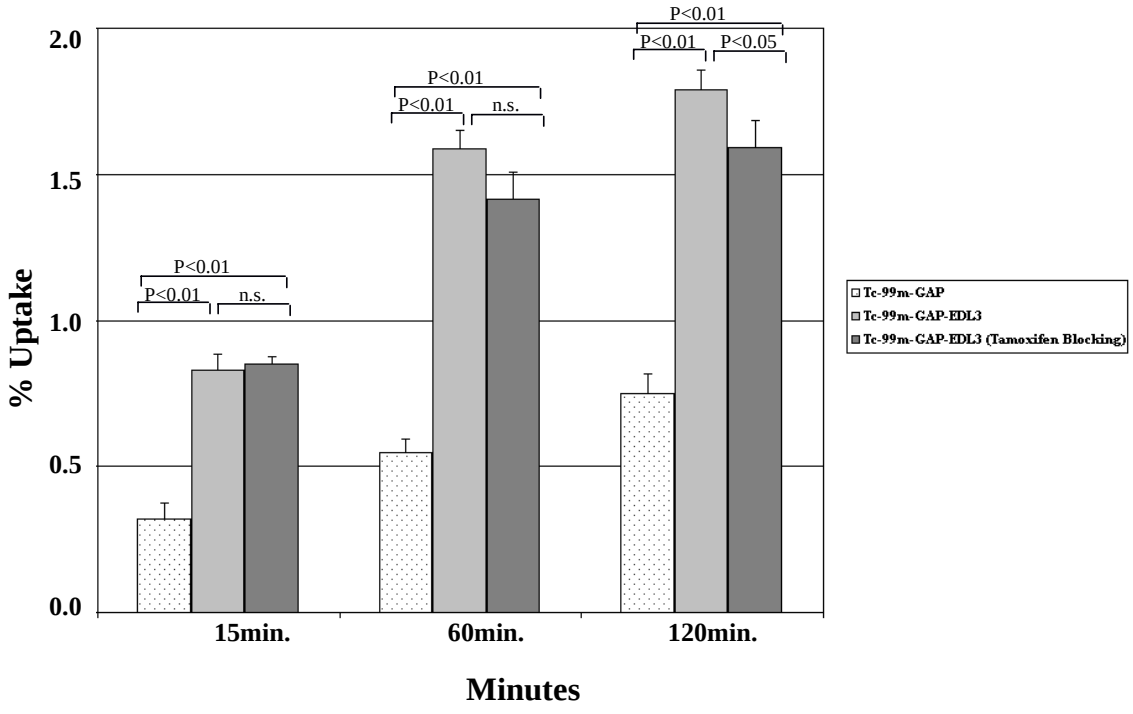
99mTc-GAP-EDL
DES+
99mTc-GAP-EDL
MCF-7, ER(+)
T47D, ER(+)

Fig. 5.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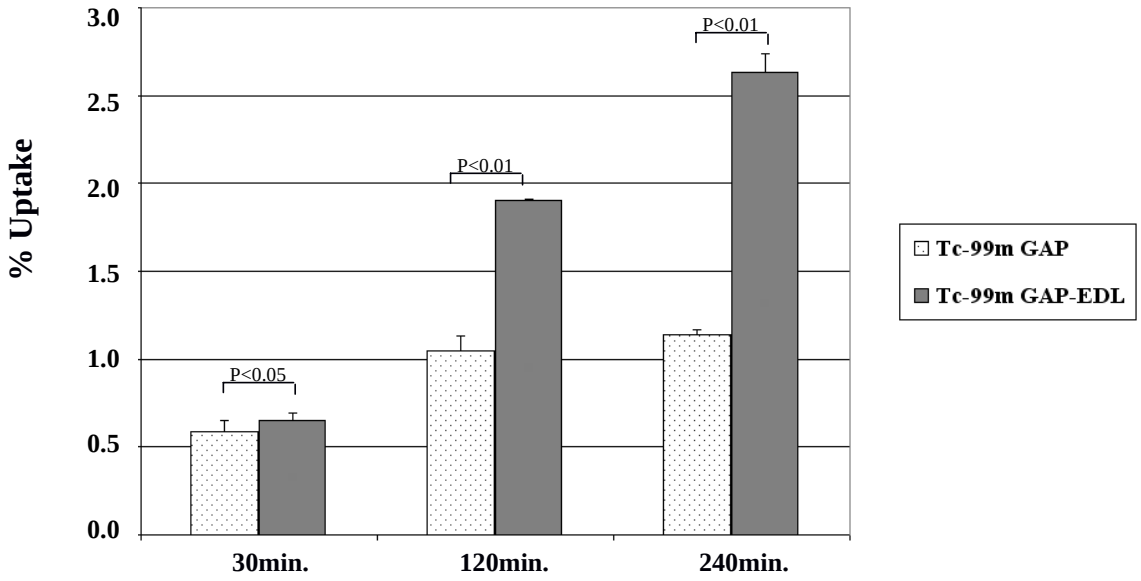
1.5
1.0
0.5
0.0
% Uptake
15min.
60min.
120min.
Minutes
Tc-99m-GAP
Tc-99m-GAP-EDL3
Tc-99m-GAP-EDL3 (Tamoxifen Blocking)

Fig. 6.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% Uptake
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30min.
120min.
240min.
Tc-99m GAP
Tc-99m GAP-EDL

Fig. 7.

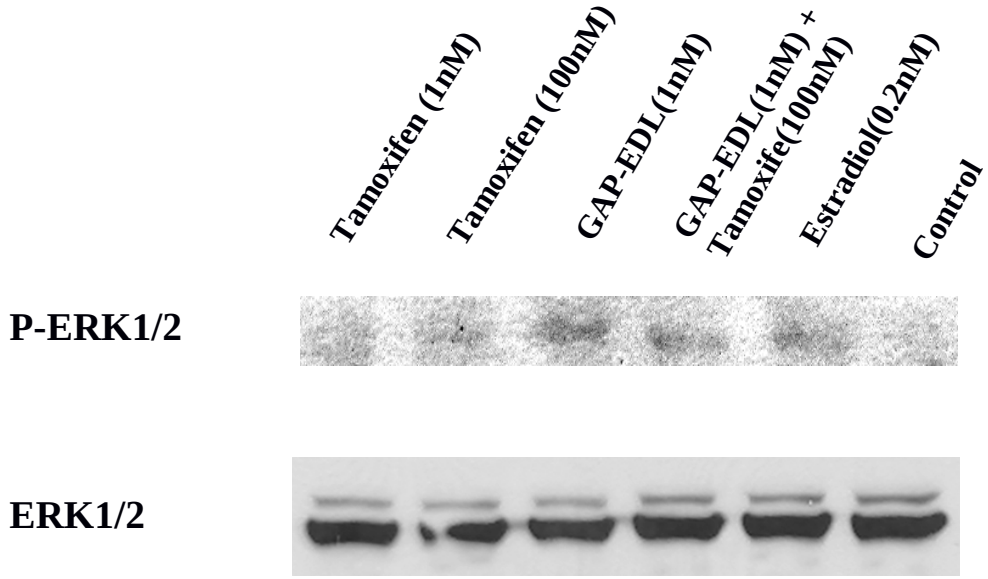

Tamoxifen (1nM)
Tamoxifen (100nM)
GAP-EDL(1nM)
GAP-EDL(1nM) + Tamoxife(100nM)
Estradiol(0.2nM)
Control
P-ERK1/2
ERK1/2

Fig. 8.

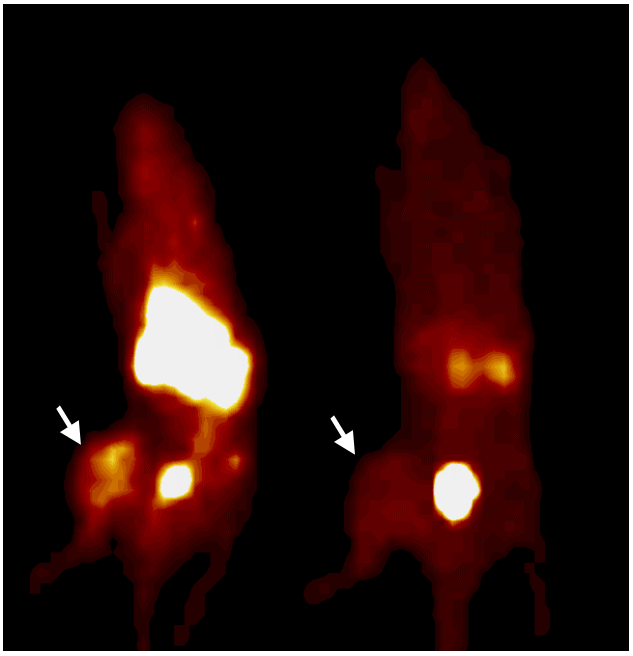
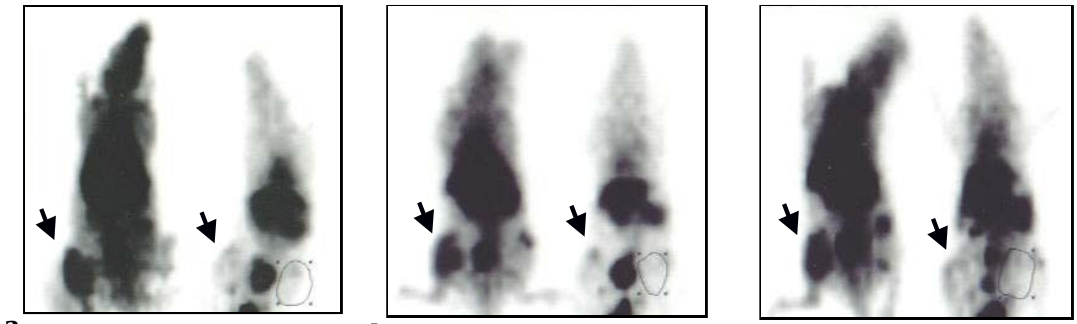


Fig. 9.



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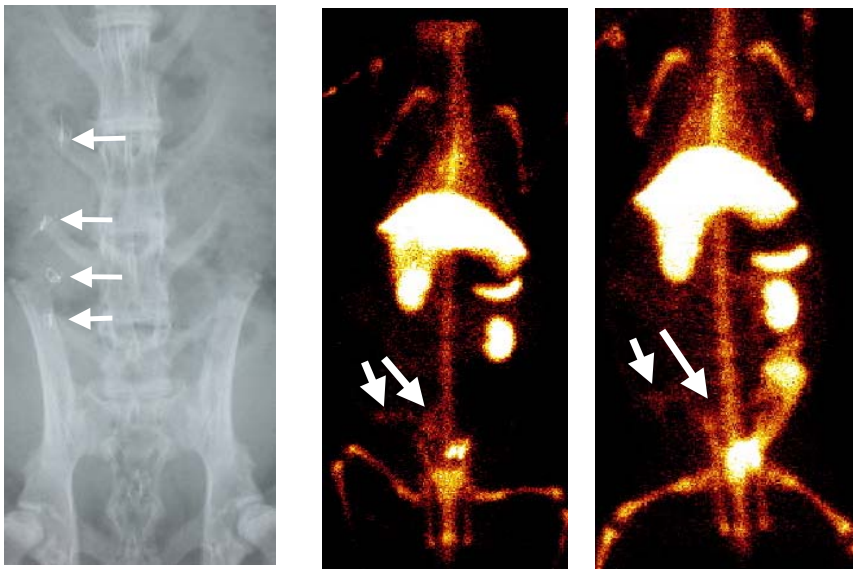

a **b** **c**

Fig. 11.

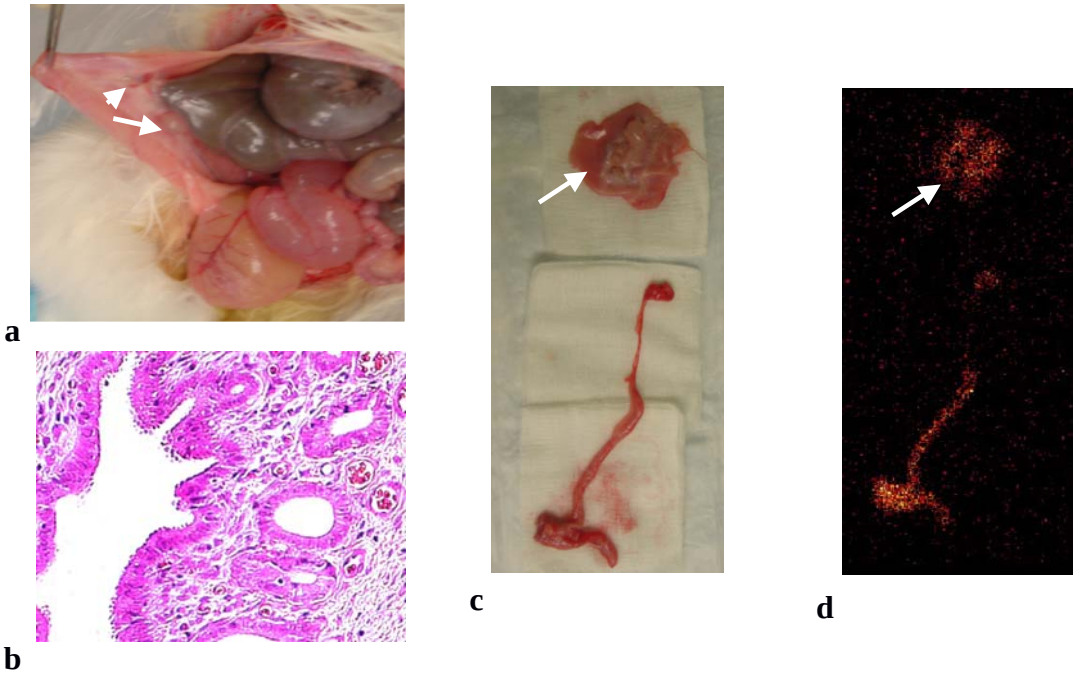

Fig. 12.

Table 1. Biodistribution of ^{99m}Tc-GAP in Breast Tumor Bearing Rats

% of injected dose per gram of tissue weight (n=3/time interval, iv)[1]

	30 MIN	2 Hours	4 Hours
BLOOD	1.71 ±0.05	0.92 ±0.23	0.59 ±0.01
HEART	0.43 ±0.05	0.27 ±0.06	0.18 ±0.01
LUNG	0.85 ±0.01	0.47 ±0.11	0.33 ±0.01
LIVER	3.45 ±0.34	3.53 ±0.33	2.88 ±0.23
SPLEEN	1.63 ±0.07	1.54 ±0.39	1.07 ±0.09
KIDNEY	10.14 ±0.74	13.16 ±4.09	11.70 ±0.76
INTESTINE	0.29 ±0.11	0.27 ±0.04	0.15 ±0.07
UTERUS	0.45 ±0.02	0.39 ±0.07	0.19 ±0.02
MUSCLE	0.13 ±0.02	0.07 ±0.01	0.06 ±0.02
TUMOR	0.52 ±0.04	0.39 ±0.04	0.32 ±0.01
THYROID	0.65 ±0.08	0.33 ±0.11	0.34 ±0.01
STOMACH	0.41 ±0.03	0.30 ±0.09	0.17 ±0.01
T/MUSCLE	4.24 ±0.88	5.98 ±0.90	6.01 ±0.05
T/BLOOD	0.30 ±0.01	0.43 ±0.08	0.55 ±0.02
UTERUS/ BLOOD	0.26 ±0.01	0.44 ±0.15	0.32 ±0.04
UTERUS/ MUSCLE	3.64 ±0.47	6.01 ±1.40	3.52 ±0.46

1. Value represents the mean ± standard deviation of data from 3 rats.

Table 2. Biodistribution of ^{99m}Tc-GAP-EDL in Breast Tumor Bearing Rats

% of injected dose per gram of tissue weight (n=3/time interval, iv)[1]

	30 MIN	2 Hours	4 Hours
BLOOD	*2.39 ±0.02	1.23 ±0.17	*0.98 ±0.04
HEART	0.52 ±0.02	0.31 ±0.04	*0.31 ±0.01
LUNG	*1.06 ±0.03	0.60 ±0.08	*0.48 ±0.03
LIVER	*6.19 ±0.10	5.01 ±0.76	*5.33 ±0.16
SPLEEN	2.25 ±0.17	1.86 ±0.25	2.14 ±0.22
KIDNEY	8.08 ±0.44	9.55 ±1.26	12.31 ±0.05
INTESTINE	0.43 ±0.05	0.27 ±0.05	0.28 ±0.02
UTERUS	0.44 ±0.06	0.46 ±0.07	*0.50 ±0.02
MUSCLE	0.11 ±0.01	0.07 ±0.01	0.06 ±0.01
TUMOR	0.45 ±0.04	0.41 ±0.07	*0.52 ±0.04
THYROID	0.54 ±0.05	0.33 ±0.08	0.34 ±0.02
STOMACH	0.36 ±0.03	0.27 ±0.03	0.21 ±0.02
T/MUSCLE	4.04 ±0.37	5.91 ±0.41	*7.92 ±0.56
T/BLOOD	0.19 ±0.02	0.33 ±0.01	0.53 ±0.02
UTERUS/BLOOD	0.18 ±0.03	0.39 ±0.14	*0.52 ±0.03
UTERUS/MUSCLE	3.93 ±0.58	6.86 ±1.30	*7.92 ±0.56

1. Value represents the mean ± standard deviation of data from 3 rats. (* $p<0.05$ vs. ^{99m}Tc-GAP)